Peak matching for $C_{24}H_{23}OPS$ gave m/e (M^+) 390.1207; found, 390.1176; for $^{13}C^{12}C_{23}H_{23}OPS$, 391.1241; found, 391.1262.

Synthesis of *r*-1,*trans*-2(*e*),6(*e*)-Triphenyl-*cis*-3(*e*)-methyl-4-phosphorinanone (2a). Ketophosphine **5a** (0.03 g, 0.14 mmol) was placed in a sealed glass tube under N_2. This system was heated on an oil bath (200–210 °C) for 2 h. The remaining procedure was that described previously to prepare **4a**. The solid was recrystallized (CH_3CN) to give a white crystalline ketone **2a** (0.036 g, 72%), mp 210–211 °C (lit.[3] mp 210–211 °C).

Synthesis of *r*-1,*trans*-2(*e*),6(*e*)-Triphenyl-*cis*-3(*e*)-methyl-4-phosphorinanone 1-Oxide (2b). Ketophosphine **2a** (0.2 g, 0.58 mmol) was dissolved in acetone (25 mL), and this solution was cooled in ice. To this was added, dropwise with stirring, 0.12 g (0.70 mmol) of MCPA in 5 mL of dry ether. The remaining procedure paralleled that for **4b**. The residue was recrystallized (abs C_2H_5OH) to give 0.11 g (52.6%) of **2b**, mp 289–291 °C (lit.[3] mp 289–291 °C).

Synthesis of *r*-1,*trans*-2(*e*),6(*e*)-Triphenyl-*cis*-3(*e*)-methyl-4-phosphorinanone 1-Sulfide (2c). Ketone **2a** (0.2 g, 0.58 mmol) and sulfur (0.02 g, 0.62 mmol) were dissolved in toluene (25 mL). The reaction mixture was gently boiled for 8 h under N_2, and the remaining procedure was the same as that described for **4c**. The residue was recrystallized (absolute C_2H_5OH) to give 0.17 g (78%) of **2c**, mp 246–247 °C (lit.[3] mp 246–247 °C).

Synthesis of *r*-1,*trans*-2(*e*),6(*e*)-Triphenyl-*cis*-3(*e*),5(*e*)-dimethyl-4-phosphorinanone (6a). Ketophosphine **3a** (0.10 g, 0.27 mmol) was placed in a sealed glass tube under N_2. This was heated on an oil bath (210–215 °C) for 0.5 h. The remaining procedure was the same as that described for **4a**. The solid was recrystallized (CH_3OH) to give **6a** (0.75 g, 75%), mp 224–226 °C. Spectral data are given in Tables I–III.

A similar procedure was adopted to prepare *r*-1,*trans*-2(*e*),6(*e*)-triphenyl-*cis*-3(*e*),5(*e*)-dimethyl-4-phosphorinanone-2,6-$^{13}C_2$ (**6a**).

Peak matching for $C_{25}H_{25}OP$ gave m/e (M^+) 372.1643; found, 372.1640; for $^{13}C^{12}C_{24}H_{25}OP$, 373.1677; found, 373.1689.

Synthesis of *r*-1,*trans*-2(*e*),6(*e*)-Triphenyl-*cis*-3(*e*),5(*e*)-dimethyl-4-phosphorinanone 1-Oxide (6b). Ketone **6a** (0.04 g, 0.108 mmol) was dissolved in acetone (10 mL). To the ice-cooled solution was added, dropwise with stirring, 0.02 g (0.116 mmol) of MCPA in 3 mL of dry ether. The remaining procedure was as for **4b**. The solid was recrystallized (abs CH_3OH) to give 0.023 g (56.6%) of **6b**, mp 302–303 °C. Spectral data are given in Tables I–III.

The corresponding ^{13}C-labeled oxide, *r*-1,*trans*-2(*e*),6(*e*)-triphenyl-*cis*-3(*e*),5(*e*)-dimethyl-4-phosphorinanone-2,6-^{13}C 1-oxide, was prepared in a similar fashion.

Peak matching for $C_{25}H_{25}O_2P$ gave m/e (M^+) 388.1592; found, 388.1585; for $^{13}C^{12}C_{24}H_{25}O_2P$, 389.1625; found, 389.1646.

Synthesis of *r*-1,*trans*-2(*e*),6(*e*)-Triphenyl-*cis*-3(*e*),5(*e*)-dimethyl-4-phosphorinanone 1-Sulfide (6c). Ketophosphine **6a** (0.05 g, 0.134 mmol) and sulfur (0.0043 g, 0.134 mmol) were dissolved in toluene (15 mL). The remaining procedure was as that for **4c**. The solid mass was recrystallized (absolute CH_3OH) to give 0.03 g (56%) of **6c**, mp 291–292 °C. Spectral data are given in Tables I–III.

An identical method was utilized to prepare *r*-1,*trans*-2(*e*),6(*e*)-triphenyl-*cis*-3(*e*),5(*e*)-dimethyl-4-phosphorinanone-2,6-$^{13}C_2$ 1-sulfide (**6c**).

Peak matching for $C_{25}H_{25}OPS$ gave m/e (M^+) 404.1364; found, 404.1368; for $^{13}C^{12}C_{24}H_{25}OPS$, 405.1397; found, 405.1391.

Synthesis of *r*-1,*trans*-2(*e*),6(*e*)-Triphenyl-*cis*-3(*e*),5(*e*)-dimethyl-3,5-dideuteriophosphorinan-4-one 1-Sulfide (12). Ketone **6c** (0.03 g, 0.074 mmol) was dissolved in dry dioxane (3 mL), and to this was added deuterium oxide (6.5 mL, Aldrich Gold Label) and K_2CO_3 (0.012 g, 0.087 mmol). The mixture was boiled with stirring under N_2 for 36 h. Upon cooling, the new mixture was extracted with $HCCl_3$ (3 × 10 mL). The $HCCl_3$ layer was washed with deuterium oxide (10 mL) and dried (Na_2SO_4). Evaporation of the $HCCl_3$ left a solid mass which was recrystallized (CH_3OH) to give 0.027 g (89.4%) of the deuterated analogue **12**, mp 290–291 °C; calcd for $C_{25}H_{23}D_2OPs$, 406.1489; found, 406.1472.

Preparation of *r*-2,*trans*-6-Diphenyl-*cis*-3-methyl-4-thianone and *r*-2,*trans*-6-Diphenyl-*cis*-3-methyl-4-thianone-6-^{13}C (10). Michael addition of hydrogen sulfide to 2-methyl-1,5-diphenyl-1,4-pentadien-3-one and 2-methyl-1,5-diphenyl-1,4-pentadien-3-one-5-^{13}C (2.0 g, 8.07 mmol) in methanol (40 mL) containing sodium acetate trihydrate (4.0 g, 0.029 mol) as reported[4a] gave 0.40 g (17.33%) of the title compound, mp 151–152 °C (lit.[4a] 151–152 °C); calcd for $C_{18}H_{18}OS$, 282.1078; found, 282.1079; for $^{13}C^{12}C_{17}H_{18}OS$, 283.1112; found, 283.1112.

Acknowledgment. We gratefully acknowledge the support of the work by the National Institutes of Health, U.S.P.H.S., via a grant from the National Cancer Institute, CA 22770-03.

Nuclear Magnetic Resonance Investigation of the Spontaneous Decarboxylation of 2-Oxalopropionic Acid. 2. Species in Solution

Gregory Kubala† and A. E. Martell*

Contribution from the Department of Chemistry, Texas A&M University, College Station, Texas 77843. *Received July 28, 1981*

Abstract: The kinetics of the spontaneous decarboxylation of 2-oxalopropionic acid (OPA) to the enolate intermediate of α-ketobutyric acid (AKBA) with subsequent ketonization, and β-deuteration via enolization, have been studied by NMR in aqueous solution at 31 °C. The rate constants for the decarboxylation of the fully protonated, monoprotonated, and fully deprotonated species of OPA were found to be 1.67×10^{-5} s^{-1}, 13.5×10^{-5} s^{-1}, and 7.75×10^{-5} s^{-1}, respectively. The rate constant for the ketonization of the intermediate was found to be 3.25×10^{-4} s^{-1} while the rate constant for the enolization of OPA was found to 2.70×10^{-4} s^{-1}. The ketonization and enolization processes exhibited specific acid catalysis and the second-order rate constants were found to be 1.60×10^{-1} M^{-1} s^{-1} and 1.20×10^{-1} M^{-1} s^{-1}, respectively. The first pK_a of OPA, involving the carboxyl adjacent to the keto function, was found to be 1.75, while the second pK_a for the remaining carboxyl group was determined to be 4.18. In D_2O the pK's were calculated as 2.38 and 4.50, respectively. Under the reaction conditions employed the hydrate species exists in appreciable concentrations at low pH while the concentration of the enol species was not significant.

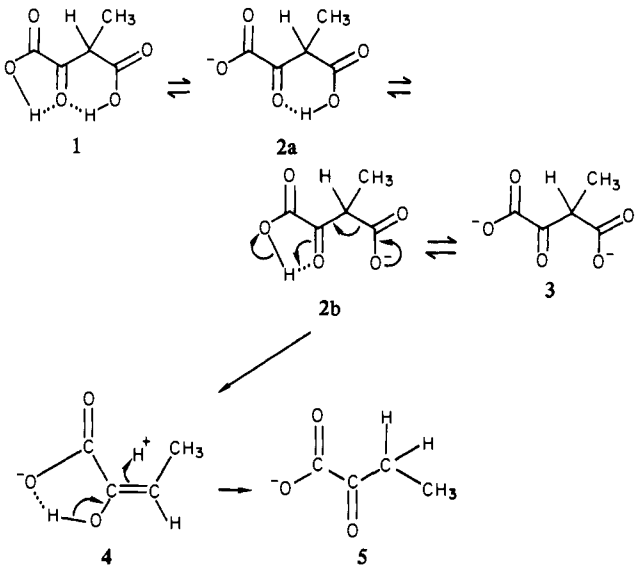
The spontaneous,[1-9] metal-catalyzed,[5-18] and enzymatic[19] decarboxylation of α-keto diacids in which the second carboxyl function is located at the β carbon, along with the corresponding enolization[20-22] and dehydration[20,21] reactions, have been the subject of detailed kinetic studies and still continue to be of widespread scientific interest. Substrates which have been involved in these studies are oxaloacetic acid (OAA), dimethyloxaloacetic

† Abstracted in part from a dissertation to be submitted by Gregory Kubala to the Faculty of Texas A&M in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(1) Ochoa, S. *J. Biol. Chem.* **1948**, *174*, 115.
(2) Gelles, E. *J. Chem. Soc.* **1956**, 4736.
(3) Sakkab, N. Y.; Martell, A. E. *J. Am. Chem. Soc.* **1976**, *98*, 5285.
(4) Steinberger, R.; Westheimer, F. H. *J. Am. Chem. Soc.* **1949**, *71*, 4158.
(5) Kornberg, A.; Ochoa, S.; Mehler, A. H. *J. Biol. Chem.* **1948**, *174*, 159.
(6) Krebs, H. A. *Biochem. J.* **1942**, *36*, 303.

0002-7863/81/1503-7609$01.25/0

Scheme I



acid (DMOAA), fluorooxaloacetic acid (FOAA), and 2-oxalopropionic acid (3-methyl-2-oxobutanedioic acid, OPA). Decarboxylation of these substrates results in the formation of pyruvic, α-ketoisovaleric, fluoropyruvic, and α-ketobutyric (AKBA) acids, respectively. The mechanism proposed for these decarboxylation reactions is illustrated in Scheme I. There are several points of interest to be considered with respect to this mechanism, the first of which is the dependence of the rate constant k_1 on pH. The most rapid pathway for the decarboxylation of OAA and DMOAA occurs through the formation of the monoanion **2b**, whereas the diprotonated and the fully ionized forms tend to decompose at slower rates.[4] The generally accepted explanation for this experimental evidence is that electron density (i.e., a negative charge) must be available for the carboxyl group which is to be cleaved for the required electron shift to occur and that the existence of the hydrogen bond between the oxalyl carboxyl and the α-carbonyl oxygen assists in the electron shift. Until recently, most of the experimental work accomplished has been concerned with only the observed rate constants of decarboxylation with respect to the degree of protonation of OAA and its analogues. This was due to the fact that there has been controversy as to the nature of the substrates in solution, and considerable effort has been expended in attempts to define the concentrations of keto, enol, and hydrate species present during decarboxylation.[11,20,23-26]

In the early studies of OAA, involving the use of ultraviolet spectroscopy,[27-29] the enol concentration was estimated by employing certain assumptions about the species in solution and the values of their extinction coefficients. Sakkab and Martell[3] questioned the validity of these postulates and Kokesh[26] subsequently measured keto, enol, and hydrated forms of oxaloacetic acid and its mono- and dianions with nuclear magnetic resonance. These NMR studies revealed that the hydration of OAA has no effect on the calculated enol concentration in most cases as long as the excitation coefficients of the keto and hydrate species are much smaller than that of the enol. Kumler et al.[30] were the first to successfully apply NMR spectroscopy in calculating an equilibrium constant for the keto–enol tautomerization. However, in addition to the keto and enol tautomers they reported an unknown species, which was later identified by Pogson and Wolfe[20] as the hydrate. It was reasoned that both the hydrate and enol forms were inactive toward decarboxylation and that the decarboxylation proceeds through the keto form only, but it was also pointed out[3] that the concentration of hydrate and enol species must be known if an accurate rate constant was to be determined for decarboxylation. The β protons of OAA in D_2O solutions deuterate and as a result the individual molecular species in OAA solutions cannot be monitored by NMR during decarboxylation. Sakkab and Martell[3] introduced 2-oxalopropionic acid as a substrate for decarboxylation studies since the β-methyl group provides a direct NMR probe. Also, because it has the ability to enolize, it is a better model for OAA than DMOAA.

The initial studies[3] on the decarboxylation of OPA and the subsequent ketonization of the AKBA intermediate were limited to two very narrow pH ranges (pD 1.30–1.92, unbuffered; pD 4.44–4.75, deuterioacetate buffer). The present work was carried out to obtain a complete pD–rate profile, which is necessary to determine the degrees of protonation of the reactive species and the extent of interaction between keto, enol, and hydrate forms. A second goal is to elucidate the nature of the subsequent ketonization step and to calculate an accurate value of the rate constant for this reaction. Since the kinetic experiments were carried out in D_2O, a third objective is to determine a rate of enolization and β-proton exchange of OPA with subsequent deuteration of the β carbon. These investigations are an important first step in a much broader investigation involving both pyridoxamine catalysis of decarboxylation of carboxyl groups substituted on the β-carbon atoms of α-keto acids, and of pyridoxal catalysis of decarboxylation of remote carboxyl groups of α-amino acids such as aspartic acid. A full understanding of the mechanism of decarboxylation of these substrates is considered a prerequisite of the interpretation of the vitamin B_6 catalyzed systems, which are of importance for comparison with the corresponding enzyme-catalyzed biological processes. Schiff bases of OAA and its analogues with pyridoxamine are probable intermediates for the decarboxylation of the corresponding aminodicarboxylic acids and may be formed by transamination of the latter.

Experimental Section

Materials. Diethyl 2-oxalopropionate was obtained from the Aldrich Chemical Co. along with D_2O (99.8% pure), 20% DCl in D_2O (99+% pure), and 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt hydrate, α-Ketobutyric acid was purchased from Mann Laboratories and was used without further purification. The ionic strength of the D_2O solutions was controlled by the addition of certified ACS grade potassium chloride obtained from Fisher. Chloroform-*d* (99.8% pure), acetone-d_6 (99.8% D), and tetramethylsilane (Me_4Si) were obtained from Merck.

Synthesis and Characterization of OPA. 2-Oxalopropionic acid was prepared by acid hydrolysis of diethyl oxalopropionate according to Galegov's method,[31] with modified workup and purification of the product. After 24 h of hydrolysis, dry air was blown into the reaction vessel until a colorless solid appeared. The solid material was placed

(7) Pedersen, K. J. *Acta Chem. Scand.* **1952**, *6*, 285.
(8) Kosicki, G. W.; Lipovac, S. N. *Can. J. Chem.* **1964**, *42*, 403.
(9) Dummel, R. J.; Berry, M. H.; Kun, E. *Mol. Pharmacol.* **1971**, *7*, 367.
(10) Steinberger, R.; Westheimer, F. H. *J. Am. Chem. Soc.* **1951**, *73*, 429.
(11) Nossal, P. M. *Aust. J. Exp. Biol. Med. Sci.* **1949**, *27*, 143, 312.
(12) Gelles, E.; Salama, A. *J. Chem. Soc.* **1958**, 3689.
(13) Speck, J. F. *J. Biol. Chem.* **1949**, *178*, 315.
(14) Munakata, M.; Matsui, M.; Tabushi, M.; Shigematsu, T. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 114.
(15) Bontchev, P. R.; Michaylova, V. *J. Inorg. Nucl. Chem.* **1967**, *29*, 2945.
(16) Covey, W. D.; Leussing, D. L. *J. Am. Chem. Soc.* **1974**, *96*, 3860.
(17) Raghaven, N. V.; Leussing, D. L. *J. Am. Chem. Soc.* **1976**, *98*, 723.
(18) Rund, J. V.; Claus, K. G. *J. Am. Chem. Soc.* **1967**, *89*, 2256.
(19) Krampitz, L. D.; Werkman, C. W. *Biochem. J.* **1974**, *35*, 595.
(20) Pogson, E. I.; Wolfe, R. G. *Biochem. Biophys., Res. Commun.* **1972**, *46*, 1048.
(21) Emly, M.; Leussing, D. L. *J. Am. Chem. Soc.* **1981**, *103*, 628.
(22) Bruice, P. Y.; Bruice, T. C. *J. Am. Chem. Soc.* **1978**, *100*, 4793.
(23) Meyer, K. H. *Ber.* **1912**, *45*, 2843.
(24) Hautsch, A. *Ber.* **1915**, *48*, 1407.
(25) Gelles, E.; Hay, R. W. *J. Chem. Soc.* **1957**, 3673.
(26) Kokesh, F. C. *J. Org. Chem.* **1976**, *41*, 3593.
(27) Banks, B. E. *J. Chem. Soc.* **1962**, 63.
(28) Banks, B. E. *J. Chem. Soc.* **1961**, 5043.
(29) Kosicki, G. W. *Can. J. Chem.* **1962**, *40*, 1280.
(30) Kumler, W. D.; Kun, E.; Schoolery, J. N. *J. Org. Chem.* **1962**, *27*, 1165.
(31) Galegov, G. A. *Biokhimiya (Moscow)* **1961**, *26*, 635.

under vacuum at room temperature for 1 day and was then dissolved in a minimal amount of dry ether. A threefold volume of dry benzene was added, and the ether was driven off by mild heating. The OPA separated from the solution on cooling, was recrystallized from benzene, and was stored in a vacuum desiccator over $CaCl_2$ and KOH. The yield of product melting at 144 °C was 21% of the theoretical amount.

An acid–base titration of OPA yielded an equivalent weight of 145.2 g/mol, compared to a calculated value of 146.09 g/mol. Infrared and NMR spectra indicated that the diester had been cleaved to the diacid while ^{13}C and ^1H NMR of OPA in acetone-d_6 (with chloroform-*d* and Me_4Si as internal standards) indicated the crystalline OPA to be in its keto form on the assumption that keto–enol tautomerization is slow in acetone.

OPA was dissolved in aqueous 0.10 M KCl, and a potentiometric titration was performed with 0.100 M NaOH solution. For prevention of interference resulting from decarboxylation, the titration method described by Tate et al.[32] was employed. A computer program developed by Dr. R. J. Motekaitis[33] was used to calculate the pK_a's.

Measurements. The pH values of the solutions used in the kinetic experiments were measured with a Beckman Research pH meter fitted with a Sargent-Welch miniature combination glass electrode. The pD values of the deuterium oxide solutions were calculated by adding 0.40 to the observed reading on the meter[34] which was standardized with strong acid and base so as to read hydrogen ion concentration directly. ^1H NMR spectra of 0.8 M OPA solutions were obtained with a Varian HA 100 NMR spectrometer, and chemical shifts are reported in parts per million with respect to 3-(trimethylsilyl)-1-propanesulfonic acid. The ambient temperature of the probe was 31 ± 1 °C. ^{13}C NMR spectra were measured with a JEOL PFT-100 NMR spectrometer. The ultraviolet absorption spectra were measured in 1.00-cm matched quartz cells with a Cary Model 14 recording spectrophotometer. The infrared spectra were obtained as Nujol mulls with a Beckman IR8 infrared spectrophotometer.

Kinetic measurements were conducted on unbuffered solutions in order to eliminate any possible catalytic effect that buffers would have on decarboxylation, ketonization, or enolization. The absence of buffers allows the accurate determination of specific rate constants of decarboxylation for the diprotonated, monoprotonated, and unprotonated OPA species and also makes possible the calculation of the second-order rate constants of specific acid catalysis of ketonization and enolization. The absence of buffering agents in the system resulted in some drift in the pD in the weakly acidic regions during the course of the reaction. Error in kinetic values due to this drift was minimized by using initial rates. All measurements were taken before the shift in pD became significant.

Treatment of NMR Data. The method previously described by Sakkab and Martell[3] was employed to follow the decarboxylation of OPA, the formation of the enol intermediate **4**, and the enol–keto conversion of the latter to **5**. These processes follow first-order kinetics as determined by direct integration of the β-CH_3 signal of OPA and of the intermediate (see Results). The β-CH_3 signal persists throughout the course of the reaction; however when CO_2 is lost, the methyl resonance of OPA disappears while the AKBA methyl resonance appears. In addition, the α-CH signal was monitored to provide rates of enolization of the keto form of OPA. The rate of disappearance of the α-CH signal of OPA is the sum of the rates of decarboxylation and of enolization. The results obtained for the rates of decomposition of OPA were fitted to eq 1, where A_0 is the initial concentration of OPA. Values of k_1 were

$$\ln A = -k_1 t + \ln A_0 \quad (1)$$

computed on a 64K byte Z-80 microprocessor with a linear least-squares method. The rates for the subsequent enol–keto tautomerization were calculated from the solution of a relationship[3,35] describing two sequential first-order (A → B → C) processes, which gives the concentration of B as indicated by eq 2. Values of k_2 were computed by the use of the

$$B = A_0 \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad (2)$$

Newton–Raphson method of iteration.[36] In eq 2, A_0 is known, k_1 is known from eq 1, and B is obtained from the direct integral of the β-CH_3 resonance of the enol intermediate 4. Values for the observed rate constant for the disappearance of the OPA α-CH signal (k_{obsd}) were calculated with a first-order equation similar to eq 1 via a linear least-squares method, and the enolization rate constant of OPA (k_3) was calculated by difference:

$$k_3 = k_{obsd} - k_1 \quad (3)$$

The specific rate constants for the diprotonated, monoprotonated, and unprotonated species were calculated from the pD profile of the k_1 values by means of a program developed by Dr. K. Tatsumoto.[37]

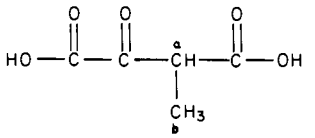
Table I. Effects of Deuterium Oxide on Proton Dissociation Constants at 25 °C

acid	pK^H	pK^D	ΔpK_a	K^H/K^D	ionic strength
OPA, pK_1[a]	1.82	2.38	0.56	3.6	0.1
maleic,[31] pK_1	1.98	2.53	0.55	3.6	<0.7
maleic,[41] pK_1	1.91	2.54	0.63	4.2	0
acetic[31]	4.73	5.25	0.52	3.3	<0.1
acetic[38]	4.74	5.21	0.47	2.91	0.05
formic[31]	3.75	4.20	0.45	2.5	<0.1
OPA, pK_2[a]	4.22	4.59	0.37	2.3	0.1
maleic,[31] pK_2	6.28	6.61	0.33	2.1	<0.1
maleic,[41] pK_2	6.33	6.71	0.38	2.4	0

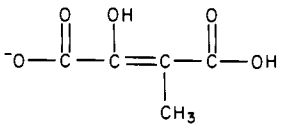
[a] 31 °C.

Results and Discussion

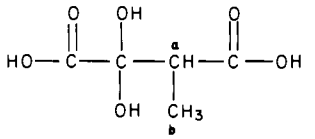
pK_a's of OPA. of OPA. There are three possible proton dissociation constants for 2-oxalopropionic acid. The first dissociation involves the carboxyl group adjacent to the keto function in **1**;



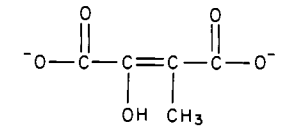
1



6



7



3a

the second dissociation applies primarily to the other carboxyl group of the keto form **2a**; and the third dissociation constant involves the enolic hydroxyl group of the species **3a** resulting from the enolization of **3**. Only the first and second pK_a's were determined. These constants overlap somewhat, but the third pK (involving the further dissociation of **3**) is quite high and should not interfere with calculation of the first two.

In aqueous solution, 2-oxalopropionic acid and its anions may exist as keto (**1**), enol (**6**), and hydrate (**7**) species. Individual dissociation constants cannot be determined for each of these species because of the lack of reliable equilibrium constants for hydration and enolization. In aqueous 0.10 M KCl at 25 °C, pK_1 = 1.75 and pK_2 = 4.18, with a standard deviation at 0.03 while at 31 °C the values are 1.82 and 4.22, respectively. Martell and Smith[38] reported values of 2.27 for pK_1 and 3.89 pK_2 of OAA at 25 °C and 0.10 ionic strength, while for DMOAA values of 1.77 for pK_1 and 4.62 for pK_2 at 25 °C and zero ionic strength were reported.

Several studies[31,39-42] have examined proton dissociation of organic acids in deuterium oxide. Their findings indicate that the pK_a's of carboxylic acids increase when placed in a deuterium medium, and the results obtained for OPA in this research are consistent with earlier observations. In 0.10 M KCl–D_2O solutions, pK_1 of OPA is 2.38 and pK_2 is 4.59. These dissociation constants in D_2O were calculated by means of a computer program which

(32) Tate, S. S.; Grzybowski, A. K.; Datta, S. P. *J. Chem. Soc.* **1964**, 1372.
(33) Harris, W. R.; Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **u1975**, *14*, 974.
(34) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.
(35) Frost, A. A.; Pearson, R. G. "Kinetics and Mechanism"; Wiley: New York, 1965; p 166.
(36) Yamada, I.; Sugie, H.; Iwata, T.; Tosaka, N. *Chem. Eng. (Tokyo)* **1966**, *4*, 375.
(37) Tatsuumoto, K.; Martell, A. E. *J. Am. Chem. Soc.* **1977**, *99*, 6082.
(38) Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum: New York, 1977; Vol. III; pp 131–132.
(39) Korman, S.; Laher, V. K. *J. Am. Chem. Soc.* **1936**, *58*, 1396.
(40) Schwarzenbach, G. *Z. Electrochem.* **1948**, *44*, 46.
(41) Hornel, J. C.; Butler, J. A. V. *J. Chem. Soc.* **1936**, 1361.
(42) Dahlgreen, G.; Long, F. A. *J. Am. Chem. Soc.* **1960**, *82*, 1303.

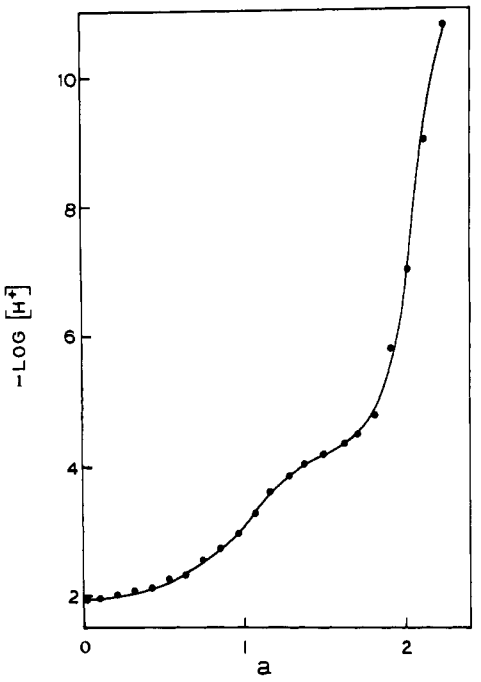
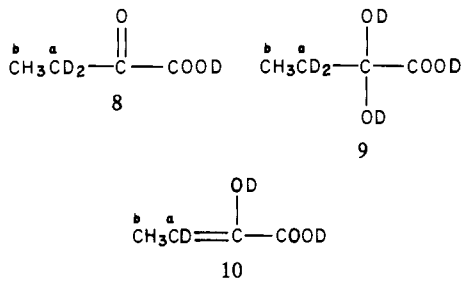


Figure 1. Potentiometric equilibrium curve of OPA at 25.00 ± 0.05 °C in 0.100 M aqueous KCl solution. The curve is a composite of measurements made on several solutions to avoid interference by decarboxylation; a = moles of base added per mole of OPA present.

varies the independently determined pK_a's slightly to provide the best fit for the pD–rate constant profile in Figure 1. The relative errors in these values are 0.14 and 0.10, respectively. Table I lists these results along with values obtained from the literature for acetic, formic, and maleic acids.

AKBA and OPA Spectra. α-Ketobutyric acid spectra were investigated by Abbott and Martell[43] and rates of ketonization of AKBA have been reported by Sakkab and Martell,[3] while enolization rates have been studied by Schellenberger and Hubner.[44] At pD 1.4, results obtained in the Abbott and Martell[43] study show that AKBA is mostly in the keto form **8** with ap-



preciable amounts of the hdyrate **9** present and that the percent of hydrate decreases with increasing pD until at pD 4 the spectrum is nearly completely that of the keto form. At pD 3.08 in the absence of buffer, the spectrum of AKBA consists of a methyl singlet (10b) of the enol form at 1.32 ppm, a methyl singlet (8b) of the keto form at 1.06 ppm, and a methyl singlet (9b) of the hydrate form at 0.64 ppm. The spectrum remains constant up to pD 7. In this pD range and in the presence of a catalytic agent such as a metal ion or a buffer, the AKBA shows evidence of dimerization.[3,43] At pD 1.66 the rate constant for ketonization of AKBA was found to be 3.15×10^{-3} min^{-1} and at pD 4.6 in a deuterioacetate buffer the rate constant was determined[3] as 5.46×10^{-2} min^{-1}. The enolization of AKBA has been shown to be subject to general base catalysis by acetate ion, and the second-order rate constant was reported[44] to be (30–50) $\times 10^{-5}$ M^{-1} min^{-1}.

(43) Abbott, E. H.; Martell, A. E. *J. Am. Chem. Soc.* **1969**, *91*, 6931.
(44) Schellenberger, A.; Hubner, G. *Chem. Ber.* **1965**, *98*, 1938.

Table II. Distribution of Keto and Hydrate Species of OPA[a]

pD	% keto	% hydrate	K_{eq}
1.55	55	45	1.2
1.59	60	40	1.5
1.87	67	33	2.0
2.04	76	24	3.2
2.47	84	16	5.3
2.61	92	8	11.5
2.76	95	5	19.0

[a] Assuming no enol content.

The spectrum of 0.8 M solutions of OPA in D_2O at pD's from 3 to 6 after 5 min from the time of mixing consists of a doublet and a quartet associated with the keto forms, **1–3**. At pD 3.08 in the absence of buffer the doublet is located at 1.14 ppm and is assigned to the $-CH_3$ (1b) resonance while the quartet is located at 2.83 ppm and is assigned to –CH (1a) resonance. Below pD 3 the spectrum consists of an overlapping pair of doublets while the quartet loses resolution and broadens. The original doublet at 1.14 ppm is one of the doublets in this pair while the second doublet is located at 1.10 ppm and is assigned to the $-CH_3$ (7b) signal of the hydrate form. The broadening of the quartet at 2.83 ppm may be due to two sets of quartets not quite superimposed upon each other. Below pD 2 there is no quartet, and the doublets are replaced by a broad singlet at about the same frequency. It is assumed that below pD 2, deuteration of the substrate at the –CH position of **1** is rapid. The fact that there are no detectable amounts of enol form in this pD range is attributed in part to low substrate concentration. If it is assumed that the ^{1}H NMR sensitivity has approximately a 5% possibility of error, this conclusion is consistent with results reported by Kokesh[26] for oxaloacetic acid, which show temperature and substrate concentration dependence of the enol signal. He did not observe an enol resonance of 0.38 M aqueous solutions of OAA at 38 °C, but with an increase in concentration to 1.14 M a signal was reported for the enol species at low and high pH. He also reported that 1.14 M solutions of the OAA monoanion at 38 °C gave no enol signal, but a braod peak attributed to the enol form was detected at 4 °C. Also, in the present studies hydrate formation of OPA was observed below pD 3.0 in 0.10 M KCl–D_2O solutions in the absence of buffer. Sakkab and Martell[3] earlier reported detectable amounts of hydrate formation for OPA, and Pogson and Wolfe[20] also noted the hydrate species of OAA. In the present studies the amount of the hydrate species of OPA detected increased as the pD was lowered. Table II lists the various pD's at which the hydrate species was observed along with the relative amounts of keto and hydrate species. This behavior is consistent with results for OAA reported by Kokesh[26] and Emly and Leussing.[21] Since interconversion between these two forms is rapid,[3,30] the conditional equilibrium constant, K_{eq}, as defined by eq 4 can be determined. These equilibrium constants are also reported in Table II.

$$K_{eq} = [\text{keto}]/[\text{hydrate}] \quad (4)$$

Decarboxylation. Five minutes after mixing, the spectrum of OPA in D_2O at pD 3.08 consisted of a doublet at 1.14 ppm and a quartet at 2.83 ppm. As the reaction proceeded, the intensity of the quartet decreased and then disappeared. The doublet collapsed to a singlet, lost intensity, and eventually was no longer detectable. While these changes occurred, a doublet appeared at 1.06 ppm which quickly collapsed to a singlet and gained intensity during the course of the reaction. At 1.32 ppm, a broad singlet appeared and increased in intensity for a short time, stabilized, then gradually weakened, and finally vanished. Late in the course of the reaction a singlet appeared at 0.64 ppm.

The behavior described above is consistent with Scheme II which indicates deuteration of the single β proton of OPA through an enolization process, with simultaneous decarboxylation to form the enol of AKBA, which subsequently ketonizes the to keto and hydrate froms. The doublet (1b) at 1.14 ppm and the quartet (1a) at 2.83 ppm are assigned to the keto form of OPA. The singlets at 1.32 ppm (10b), the singlet at 1.06 ppm (8b), and the

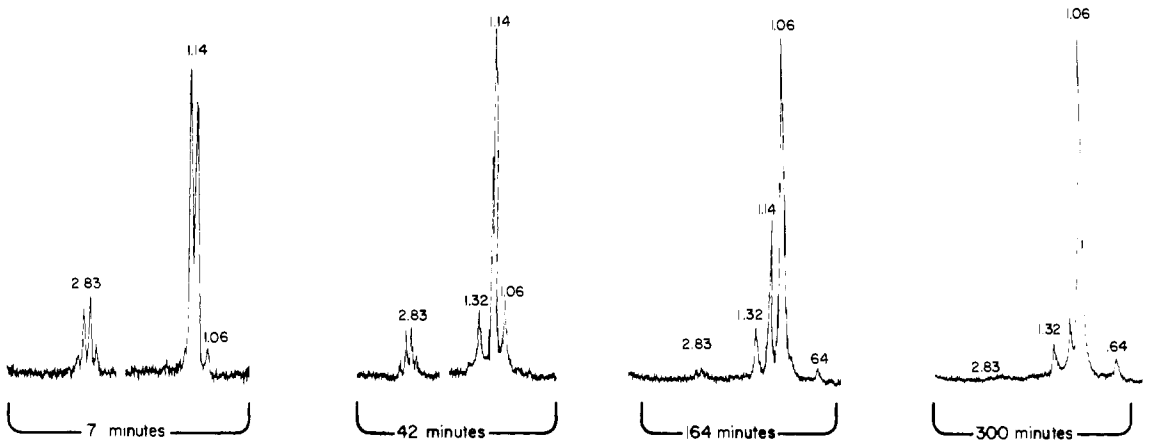
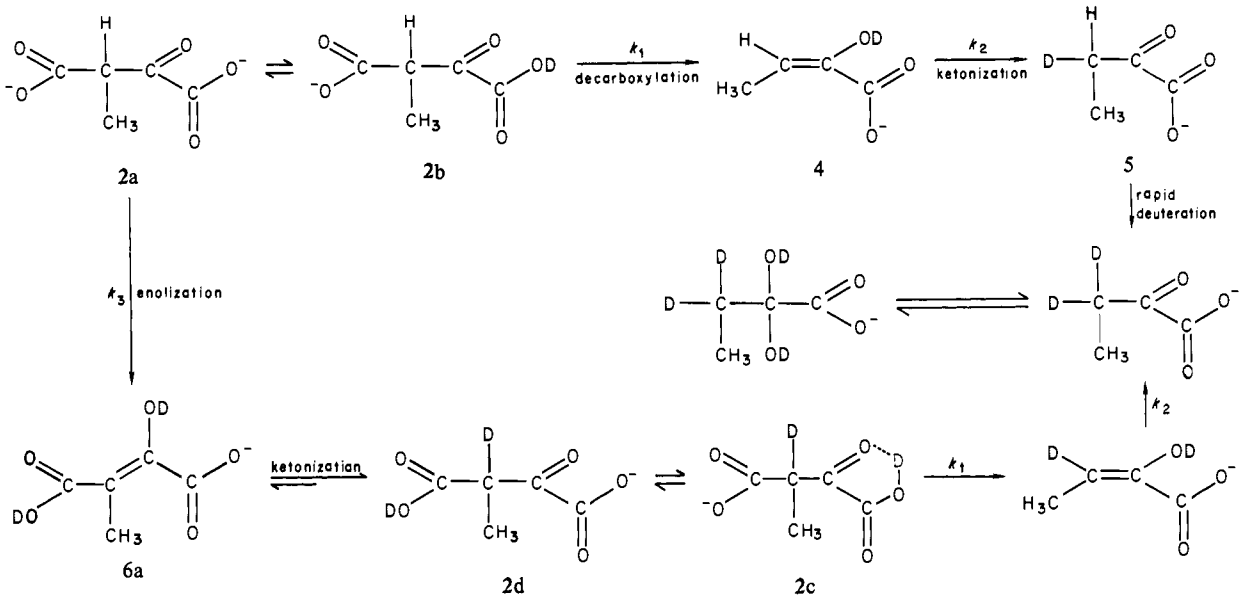


Figure 2. Nuclear magnetic resonance spectra as a function of time indicating decarboxylation and β-deuteration of OPA and ketonization of decarboxylation product in a 0.100 M KCl-D_2O solution at pD 3.08 and 31 ± 1 °C.

Scheme II



singlet at 0.64 ppm (9b) are assigned to the enol, keto, and hydrate forms of AKBA, respectively. The changes in the NMR spectra during the course of the reaction are illustrated in Figure 2.

The first-order rate constants for the decarboxylation of OPA were determined by direct analysis of the integrated intensities of the β-CH_3 signals. It was assumed that the isotope effect was minimal and that the rate constant for decarboxylation was not affected appreciably by deuteration at the β-carbon atom. The rate constant k_1 for the decarboxylation of the keto form of OPA was evaluated with the aid of eq 1 through the use of a linear least-squares calculation. The simple first-order equation is justified since the reaction conditions and procedures employed in the present work apparently have eliminated detectable amounts of the inert enol species of OPA and since rapid equilibrium exists between the active keto and the inert hydrate species. The values obtained for k_1 at 18 pD values are listed in Table III. A graphical representation of the pD profile of k_1 is given in Figure 3.

The observed first-order rate constants for decarboxylation for the overall reaction ($k_{1,obsd}$) are related to the individual or specific rate constants for the diprotonated acid (k_{H_2A}), the monoprotonated ion (k_{HA}), and the deprotonated bivalent ion (k_A):

$$k_{1,obsd}[OPA]_{total} = k_{H_2A}[H_2A] + k_{HA}[HA^-] + k_A[A^{2-}] \quad (5)$$

This equation can be reduced to

$$k_{1,obsd} = k_{H_2A}\alpha_{H_2A} + k_{HA}\alpha_{HA} + k_A\alpha_A \quad (6)$$

Table III. Rate Constants for OPA Decarboxylation (k_1, $k_{1,K}$), Enolization (k_3), and AKBA Ketonization (k_2)[a]

pD	$k_1 \times 10^5$, s^{-1}	$k_{1,K} \times 10^5$, s^{-1}	$k_2 \times 10^5$, s^{-1}	$k_3 \times 10^5$, s^{-1}
1.06	3.4			
1.43	4.2			
1.55	4.5	8.1		360 ± 22
1.59	4.9	8.1		330 ± 21
1.87	5.5	8.2		190 ± 19
2.04	6.6	8.6		135 ± 16
2.47	8.7	10.3	86	66 ± 10
2.61	9.8	10.6	80	64 ± 11
2.76	10.4	10.9	60	47 ± 8
3.08	11.5	11.5	45	42 ± 7
3.53	12.5	12.5	39	32 ± 6
3.94	12.3	12.3	34	28 ± 6
4.22	11.7	11.7	32	27 ± 4
4.58	10.5	10.5	33	27 ± 5
4.83	9.9	9.9	32	24 ± 4
5.11	9.0	9.0	32	26 ± 4
5.31	8.7	8.7	32	23 ± 4
5.68	8.3	8.3	32	26 ± 4

[a] 0.1 M KCl solutions at 30 °C. The rate constants k_1, $k_{1,K}$, and k_2 have relative errors of 0.5, 0.5, and 4, respectively.

where α is the fraction of each species of OPA present. If the pD's and the pK_a's are known, the fraction of formation of each species can be calculated from

$$\alpha_{HA} = (10^{pK_1-pD} + 1 + 10^{pD-pK_2})^1 \quad (7)$$

$$\alpha_A = \alpha_{HA} \times 10^{pD-pK_2} \quad (8)$$

$$\alpha_{H_2A} = \alpha_{HA} \times 10^{pK_1-pD} \quad (9)$$

These equations are derived from the five equations which define pK_1, pK_2, α_{H_2A}, α_{HA}, and α_A. An iterative computer calculation of k_{H_2A}, k_{HA}, and k_A was carried out to provide the best fit for data points illustrated in Figure 2. The values obtained are 1.67×10^{-5} s^{-1} for k_{H_2A}, 13.5×10^{-5} s^{-1} for k_{HA}, and 7.75×10^{-5} s^{-1} for k_A. These specific rate constants are comparable in magnitude but show a somewhat greater variation than the values reported for k_{H_2A} and k_{HA} by Sakkab and Martell.[3] The present values, however, are considered more reliable because they were obtained from data taken over a much wider pH range than was done previously. Previous research on OAA[14] has resulted in a similar pD–rate profile, indicating similar relative magnitudes for the specific rate constants. On the other hand, Pedersen[7] and Gelles[2] found that the monoprotonated anion of OAA decomposes 44 times faster than the undissociated form, although they reported a discrepancy between the observed and calculated rates when the OAA concentration was varied.

Below pD 3 appreciable amounts of the hydrate form of OPA exists. This being the case, the overall rate ($k_1[OPA]_{total}$) of decarboxylation can be expressed by eq 10 as the sum of the rates

$$k_1[OPA]_{total} = k_{1,H}[hydrate] + k_{1,K}[keto] \quad (10)$$

of decarboxylation associated with the keto and hydrate forms. In eq 10, $k_{1,H}$ and $k_{1,K}$ are the rate constants of decarboxylation of the hydrate and keto species, respectively. The above equation may be simplified to eq 11 by using eq 4. It is thought that the

$$k_1[OPA] = [keto][(k_{1,H}/K_{eq}) + k_{1,K}] \quad (11)$$

hydrate form is inert to decarboxylation, and this implies that $k_{1,H}/K_{eq}$ is very much less than $k_{1,K}$. When this assumption is applied to eq 11, the mathematical expression for $k_{1,K}$ reduces to

$$k_{1,K} = k_1([OPA]/[keto]) \quad (12)$$

Table III lists the values of $k_{1,K}$ calculated in accordance with eq 12.

The first-order rate constants, $k_{1,K}$, for decarboxylation are related to individual rate constants for the various protonated species of the keto form of OPA. These specific rate constants may be calculated by the iterative computer method previously mentioned. Also, the microscopic pK_a's of the keto form may be determined by slightly altering the macroscopic pK_a's in order to obtain the best fit for the values of $k_{1,K}$. The results of this calculation yield values of 6.99×10^{-5}, 13.0×10^{-5}, and 7.97×10^{-5} s^{-1} for the decarboxylation rate constants of the diprotonated, monoprotonated, and the deprotonated bivalent ions of the keto species, respectively. The first and second proton dissociation constants were found to be 2.56 and 4.67, respectively. The large difference ($\Delta pK_1 = 0.18$) in the microscopic pK_1 of the keto form and the macroscopic pK_1, which reflects the combined behavior of both keto and hydrate forms, may be attributed to the difference in the chemical environment of the carbonyl adjacent to a carbonyl in species **1** as opposed to the environment of the carboxyl adjacent to a *gem*-diol in species **7**.

The results of this investigation seem consistent with the discussion of Scheme I given above. The active monanion species **2b** is the tautomer in which the carboxylate group undergoing decarboxylation is negatively charged, thus making possible the shift of an electron pair required for the carbon–carbon bond-breaking process. In addition this species has a weak hydrogen bond between the carboxyl and the α-carbonyl oxygen, which has been attributed as aiding the electron shift that results in the formation of the enol intermediate. This tautomer probably exists as less than 1% of the total monoprotonated species in solution,

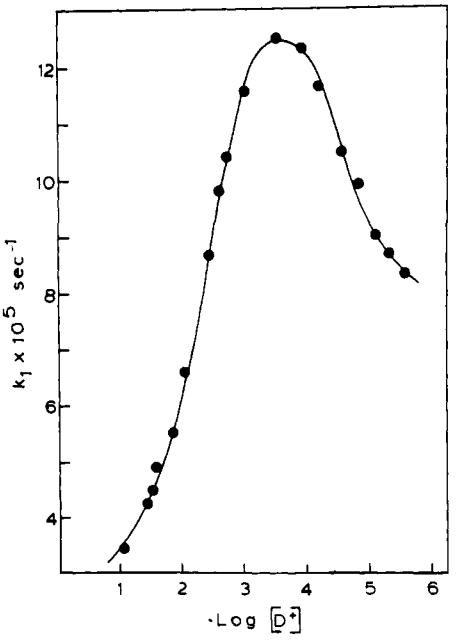


Figure 3. Graphical representation of the dependence of the decarboxylation rate constant k_1 of OPA on $-\log [D^+]$.

and it is the less reactive monoanion **2a** which comprises the bulk of the solution species. As is experimentally observed, the fully protonated species **1** is the least reactive since the electron density of the carboxyl group which is to be cleaved is tied up by protonation. The fully deprotonated species **3** is more reactive than **1**, since the negatively charged carboxyl group necessary for decarboxylation is available. The monoanion **2a**, **2b** is the most reactive, but not to the extent that might be expected because of the minor nature of species **2b**.

Ketonization. The results obtained from the spontaneous decarboxylation of OPA have been analyzed in terms of two consecutive first-order processes.[13] The choice of this model was prompted by the fact that the rate constant for the enolization of AKBA is relatively small (6.8×10^{-5} min^{-1})[44] and the rate constant for ketonization in acetate buffer is also small (5.5×10^{-2} min^{-1}).[3] In the present work the rate constant k_2 for ketonization of the enol form of AKBA was evaluated from eq 2 by the use of the Newton–Raphson method of iteration in which B is measured directly by the integration of the β-CH_3 resonance at 1.32 ppm corresponding to the enol form of AKBA; k_1 has been determined as indicated above (eq 1), and A_0 may be calculated from eq 1. The values obtained for k_2 are listed in Table III.

The ketonization step for OAA has been shown to be catalyzed by OH_3^+, OH^-, acetic acid, and acetate ion,[27] and catalysis by H^+ has been suggested for AKBA.[3] It is reasonable to assume that the ketonization of AKBA is subject to general acid, general base, specific acid, and specific base catalysis. These effects on the observed rate constant of ketonization, $k_{2,obsd}$, may be expressed by

$$k_{2,obsd} = k_2^0 + k_2^D[D^+] + k_2^{OD}[OD^-] + k_2^{HB}[HB] + k_2^B[B^-] \quad (13)$$

Under the reaction conditions of these kinetic studies, no buffer is present and OD^- concentration is minimal since all measurements are performed in acidic media. In the absence of added acid catalyst, HB, eq 13 may then be simplified to

$$k_{2,obsd} = k_2^0 + k_2^D[D^+] \quad (14)$$

This equation suggests a linear relationship between the rate constant of ketonization and deuterium ion concentration. Figure 4 graphically represents the existence of specific acid catalysis. A linear least-squares analysis yielded values of 3.25×10^{-4} s^{-1} for k_2^0 and 1.60×10^{-1} L mol^{-1} s^{-1} for K_2^D. The assumption that general acid and general base catalysis may occur in this system

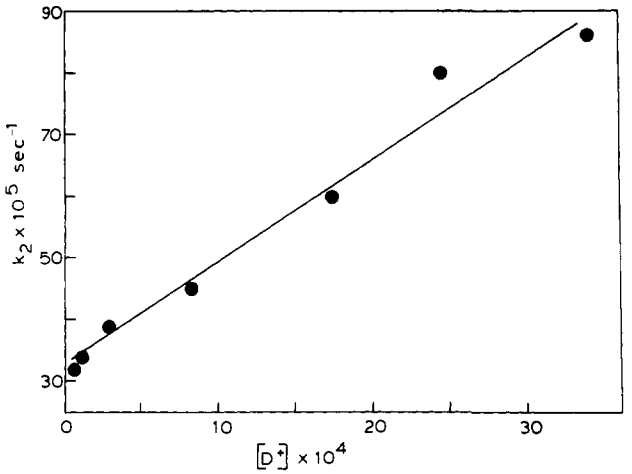


Figure 4. Graphical representation of specific acid catalysis for the ketonization of the AKBA enol intermediate in 0.100 M KCl–D_2O solutions at 31 ± 1 °C.

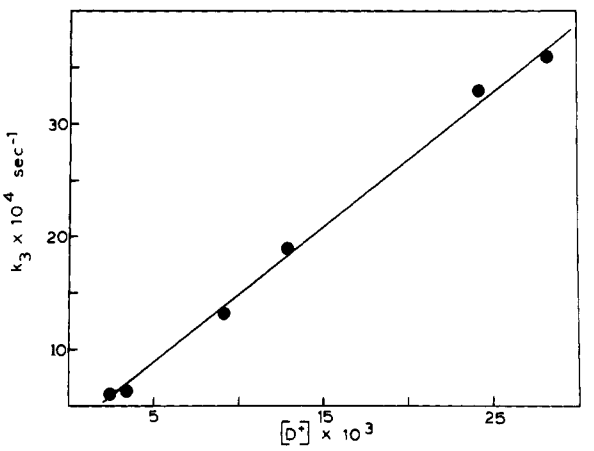


Figure 5. Graphical representation of specific acid catalysis exhibited by the rate constant, k_3, for the enolization and β-deuteration of OPA in 0.100 M KCl–D_2O solutions at 31 ± 1 °C.

is supported by the fact that the rate constant observed by Sakkab and Martell[3] of 9.10×10^{-4} s^{-1} at pD 4.60 in deuterioacetate buffer in 2.5 times larger than the rate constant, 3.33×10^{-4} s^{-1}, determined in this study in the absence of buffer at pD 4.58.

Enolization. The enolization of OPA is competitive with decarboxylation, and the disappearance of the quartet at 2.83 ppm assigned to the β proton of the keto form of OPA is the result of both these processes, as is shown in Scheme II. The NMR measures the depletion of species **2a** through decarboxylation and enolization. The fact that species **6a** is not observed by NMR, while **2a** and **2c** are, means that ketonization of **6a** is rapid and cannot be measured. The rate constant k_3 for the deuteration of the β carbon of OPA may be calculated by eq 3. It was found that the disappearance of the signal follows simple first-order behavior and may be evaluated in a manner similar to the one used in the analysis of decarboxylation. Determination of k_{obsd} for the summation of the decarboxylation and deuteration reactions makes possible the calculation of k_3, since the values of k_1 have been established. The values thus obtained for k_3 are listed in Table III.

On the basis of the behavior reported for OAA,[21,22,27] general acid, general base, specific acid, and specific base catalysis can be assumed to exist for the deuteration of the β carbon of OPA. A treatment similar to that described for the ketonization of AKBA was employed, and the behavior observed for enolization may be mathematically expressed in terms of specific acid catalysis (eq 15). Figure 5 illustrates the observed linear relationship

$$k_3 = k_3^0 + k_3^D[D^+] \quad (15)$$

between the rate constant of deuteration and deuterium ion concentration, and a linear least-squares analysis yields values of 2.70×10^{-4} s^{-1} for k_3^0 and 1.20×10^{-1} l mol^{-1} s^{-1} for k_3^D.

Summary

Research studies on the reaction kinetics of α-keto diacids in which the second carboxyl function is located in the β carbon have been advanced considerably by the application of NMR to such systems. The present findings indicate (1) the NMR method makes possible the evaluation of individual rates of simultaneous reaction involving decarboxylation, ketonization, and β-deuteration; (2) with equilibrium measurements of acid dissociation constants, the specific rate constants for decarboxylation of various protonated keto forms of OPA have been evaluated, giving the relative reactivies monoanion > bivalent anion > neutral (acid) form; (3) the ability of the neutral form to decarboxylate is further depressed by an increase in hydrogen ion concentration since greater concentrations of the diprotonated species exist in the inert hydrate form as the pH is lowered; and (4) ketonization of AKBA and β-deuteration of OPA were found to exhibit specific acid catalysis.

Acknowledgment. This work was supported by a research grant, No. AM-11694, from the National Institute of Arthritis, Metabolic and Digestive Diseases, U.S. Public Health Service.

Synthesis of 3,6-Disulfonated 4-Aminonaphthalimides

Walter W. Stewart

Contribution from the National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received February 2, 1981*

Abstract: 3,6-Disulfonated 4-aminonaphthalimides are stable, highly fluorescent, water-soluble compounds. A general synthesis of these compounds is presented: a primary amine is condensed in aqueous acid with 4-amino-3,6-disulfonaphthalic anhydride, and the product is isolated as its crystalline potassium, sodium, or lithium salt. The anhydride, whose preparation is described in detail, is interesting because it is stable indefinitely in boiling water and crystallizes readily when the solution is cooled. Because of their intense yellow-green fluorescence, solubility in water, and ability to be bound to cells and tissues, two of these 4-aminonaphthalimides have proved useful as biological tracers, and their synthesis is described in detail.

I report here a general route to an interesting group of compounds—3,6-disulfonated 4-aminonaphthalimides. The synthesis proceeds through amino anhydride **3**, a remarkable compound with several unusual properties. This anhydride, which is easily accessible, is stable indefinitely in boiling water. It condenses easily in aqueous solution with a wide range of primary amines, but not with its own amino group. A variety of N-substituted naphthalimides are conveniently prepared from the an-